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Multistage Carcinogenesis in the Urinary Bladder

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The induction of cancer of the urinary bladder is a multi-stage process involving multiple exogenous and endogenous factors. Based on the classical initiation-promotion model, we have used *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) as initiator and sodium saccharin (SAC) or tryptophan as promoters. These latter chemicals have the properties expected of promoters: induction of hyperplasia, reversibility and nonmutagenicity. Also, tumors were induced whether the promoter was administered immediately after FANFT or beginning 6 weeks after FANFT was discontinued, but no tumors resulted if either promoter was given without initiation with FANFT. Factor(s) present in normal urine also are involved in the promotion process, in addition to the role of urine as a carrier of carcinogens. However, administration of SAC to animals with a rapidly proliferating bladder mucosa, induced by ulceration, pellet insertion, or *in utero*, resulted in bladder tumor induction, even without prior initiation with FANFT. To better understand the complex interaction of the multiple variables in bladder carcinogenesis, a stochastic computer model has been formulated based on long-term carcinogenicity and tissue kinetic studies *in vivo*. This model indicates the importance of cell proliferation and the development of hyperplasia in carcinogenesis.

Urinary Bladder Cancer Induced by FANFT

Several chemicals have been identified which induce bladder cancer in humans and in experimental animals (*1,2*). Several of these, such as 2-acetylaminofluorene (AAF), induce tumors of other tissues in experimental animals, particularly the liver and breast. In the 1960's chemicals were identified which were bladder specific carcinogens, including *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) administered in the drinking water (*3*), *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) administered in the diet (*4*) or *N*-methyl-*N*-nitrosourea (MNU) administered by intravesical instillation (*5*). We have utilized the FANFT bladder cancer model. It was originally described in female Sprague-Dawley rats (*6*), but our experiments have been performed in the model developed in inbred male Fischer rats by Tiltman and Friedell (*7*).
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The details of this model and its pathogenesis have been described (*7-10*). Briefly, the bladder epithelium progresses from the normal three cell layer urothelium to a simple hyperplasia, followed by focal nodular and papillary hyperplasia, papillomas and finally carcinomas. These carcinomas produce marked hematuria and frequently become invasive. Distant metastases occur but are relatively rare in this model, and hydronephrosis is also uncommon. A dose response is observed, and a 100% incidence of bladder tumors is induced if the FANFT is administered at a dose of 0.05% of the diet or higher (*11*).

For the remainder of the experiments to be described, FANFT was administered in the diet at a dose of 0.2%. To determine the reversibility or irreversibility of the early hyperplastic changes in bladder carcinogenesis, FANFT was fed in the diet for different periods of time followed by control diet until 1 (*8*), 1½ (*10*), or 2 years (*12-14*). It was shown that FANFT administered in the diet for 6 weeks or less induced a simple hyperplasia which regressed within 2 weeks after discontinuing the diet. The bladders remained normal through 1½ years, but a few animals fed FANFT for 6 weeks developed bladder tumors by the end of 2 years. Rats fed FANFT for 8 or 10 weeks showed partial regression of the nodular and papillary hyperplasia when FANFT was discontinued in the diet, but the bladders never returned to normal. By 1 year many of these animals had developed tumors, and by 2 years most of them had developed bladder cancer. Animals fed FANFT

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for 12 weeks or more at a level of 0.2% of the diet had lesions which continued to progress even after FANFT was discontinued in the diet, and all of these animals eventually developed bladder cancer.

Initiation and Promotion in Urinary Bladder Carcinogenesis

The above studies involved the administration of a single carcinogen resulting in the induction of urinary bladder cancer. In contrast, humans are exposed to a multitude of chemicals, some of which may be bladder carcinogens. Ito and his colleagues in 1976 reported that the simultaneous administration to rats of subcarcinogenic doses of four known bladder carcinogens, BBN, AAF, FANFT, and 3, 3′-dichlorobenzidine, resulted in the induction of bladder cancer, whereas administration of any one of the chemicals at the doses used did not (*15*). In addition, they demonstrated that if the chemicals were administered sequentially rather than simultaneously bladder cancer also was induced (*16*). These studies involved the use of "complete" carcinogens.

Studies involving the use of the pellet implantation technique in mice suggested that a process similar to initiation and promotion as originally described in the mouse skin model was applicable to the urinary bladder. If a cholesterol pellet contained the 8-methyl ether of xanthurenic acid (XAE), a tryptophan metabolite, an increased incidence of bladder tumors was induced compared to the cholesterol pellet alone. However, a similar incidence of tumors was induced if the XAE was administered by subcutaneous injection rather than in the pellet, and the cholesterol pellet without XAE was subsequently implanted into the bladder (*17*).

Similar results were demonstrated using 2-aminodiphenylene oxide administered orally with a paraffin pellet in the bladder (*18*). However, the role of the pellet in these experiments is unclear (*19*).

More typical models of initiation and promotion were subsequently demonstrated for the urinary bladder by using MNU (*20*), FANFT (*12-14*), or BBN (*21, 22*) as the initiator, and sodium saccharin, sodium cyclamate, tryptophan, or phenacetin as the promoter. Described below are the studies performed in our laboratory using FANFT as the initiator and either sodium saccharin or tryptophan as the promoter.

We initially demonstrated that if FANFT was administered in the diet at 0.2% for 6 weeks, the incidence of bladder tumors was significantly increased if either sodium saccharin or D,L-tryptophan was administered, either immediately after the FANFT or after a 6-week delay period, during which time the animals were fed control diet. This 6-week delay was chosen to allow for complete evacuation of FANFT from the animal (*23*), for the simple hyperplasia present at the end of 6 weeks to regress so the bladder had returned to normal morphologically (*8*) and also to provide some evidence that the process of initiation in the urinary bladder was irreversible similar to that described in other tissues (*24, 25*). However, this dose of FANFT was not subcarcinogenic when the animals were allowed to live for 2 years. Rather, a small incidence of tumors was induced. We repeated this experiment using four weeks of FANFT as the initiator followed immediately by either sodium saccharin or L-tryptophan (*13*). Again a significant, although considerably lower incidence of tumors was induced compared to using six weeks of FANFT as the initiating dose. L-Tryptophan was used in this experiment rather than the D,L-racemic mixture since the L-form became economically available commercially.

Sodium saccharin and L-tryptophan (or its metabolites) have many of the properties known for promoters in other tissue systems. Neither compound or the metabolites or tryptophan is mutagenic in the usual *in vitro* assays (*26*), although some do cause genetic alterations such as increased recombination (*27*). These latter genetic alterations have also been found with promoting agents for other tissues (*28*). Also, both agents induce increased cell proliferation (hyperplasia) in the target organ even without prior initiation (*29, 30*). If either compound is administered alone, bladder tumors are either not induced or induced at low incidence (*1, 26*).

Effect of Regenerative Hyperplasia in Urinary Bladder Carcinogenesis

The urinary bladder epithelium is normally mitotically quiescent; the labelling index is approximately 4 per 10,000 cells (*29, 31*). Considerable evidence has accumulated suggesting that initiation occurs only during the cell cycle, not when the cell is at rest (*25*). We postulated that if the proliferation rate of the urinary bladder epithelium were increased, it may be more susceptible to the administration of an initiator such as FANFT, which under usual circumstances requires a relatively long period of administration for initiation to occur. We therefore performed the experiment shown in Table 1 (*14*). Regenerative hyperplasia was induced either by the freeze ulceration technique of Shirai et al. (*14, 32*) or by intraperitoneal injection of cyclophosphamide (*33*). With either of these techniques ulceration of the bladder epithelium occurs with consequent regenerative hyperplasia and eventual repair. The peak hyperplastic response occurs between 3 and 7 days, but remains present for approximately 3 to 4 weeks. The mitotic rate of the uri-

Table 1. Effect of regenerative hyperplasia on bladder carcinogenesis.[a]

No.	Group[b]	Effective no. of rats	Bladder tumors[c]		
			Papilloma	Carcinoma	Total
1.	Ul→FANFT→S[c]	23	0	4(17)	4(17)
2.	Ul→FANFT	22	0	0	0
3.	Ul→Cont→S	20	1(5)	5(25)	6(30)
4.	Ul	23	0	0	0
5.	FANFT→S	21	0	1(5)	1(5)
6.	FANFT	19	0	0	0
7.	S	17	0	0	0
8.	FANFT→Ul→S	22	1(5)	8(36)	9(41)
9.	FANFT→Ul	21	0	2(10)	2(10)
10.	Ul→S	21	2(10)	2(10)	4(20)
11.	CP→FANFT→S	22	3(14)	4(18)	7(32)
12.	CP→FANFT	9	0	0	0
13.	CP→Cont→S	17	2(12)	3(18)	5(30)
14.	CP	7	1(14)	0	1(14)
15.	CP→S	17	3(18)	3(18)	6(36)
16.	Cont	32	0	0	0
17.	S	20	0	0	0

[a]Data from Cohen et al. (*14*).

[b]Ul, ulcer by freezing; S, sodium saccharin as 5% of diet; FANFT, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide as 0.2% of diet for 2 weeks; CP, single IP injection of cyclophosphamide, 100 mg/kg.

[c]Numbers in parentheses represent the percentage of the rats in a group with the given lesion.

nary bladder is markedly increased during this period and nodular and papillary formation occurs. However, in both instances, the hyperplasia is completely reversible if the animals are maintained up to two years after the ulceration event; there is no formation of tumors (*14, 33, 34*). FANFT was administered for 2 weeks immediately after the ulceration event and then promoted with sodium saccharin for the remaining 102 weeks of the experiment. After either method of ulceration, bladder tumors were induced if the rats also were fed FANFT and sodium saccharin. However, bladder tumors also occurred if ulceration by either method was followed by sodium saccharin without FANFT. The results were similar whether sodium saccharin was fed immediately or after a two week delay during which time the control diet was administered. Incidences were similar to those in which FANFT was administered. Two weeks of FANFT, sodium saccharin, or the ulceration alone did not induce tumors.

These data suggest that sodium saccharin administered to an animal with a rapidly proliferating urinary bladder epithelium is carcinogenic. This is a similar situation to the previous two-generation studies with sodium saccharin in rats (*35*) and the pellet implantation experiment in mice (*36*). In both of these instances, sodium saccharin is administered to an animal with a rapidly proliferating bladder epithelium: the fetal bladder *in utero* in the two-generation study and the surgically incised and sutured bladder in the pellet implantation experiment. In the latter experiment the process of implanting a pellet into the bladder results in a regenerative hyperplasia and repair of the incision with similar tissue kinetics as those following freeze ulceration or cyclophosphamide. In the pellet implantation experiments, the pellet may be acting as a continuous stimulus for epithelial proliferation.

This experiment would also suggest that urinary bladder carcinogenesis can occur without mutation occurring. In this situation, mutation is strictly defined as a change in nucleotide content as would be detected in *in vitro* assays such as the Ames assay. However, other types of genetic alteration, such as recombination, translocation, or increased ploidyism, might be involved. Lack of mutation is supported by the findings that the incidence of tumors with ulceration followed by sodium saccharin was not increased if FANFT was administered for a period of 2 weeks immediately after the ulceration, and there was no increased incidence if ulceration was induced by cyclophosphamide versus freezing. FANFT (*37*) and cyclophosphamide (*38*) administration each result in urine with marked mutagenic activity.

The Role of Urine in Bladder Carcinogenesis

Experiments in dogs using aromatic amines as the carcinogens demonstrated the role of urine as a carrier of carcinogens (*1, 2*). This theory has been based on the ability of the kidney to concentrate various substances to higher levels in the urine than in the serum. Such concentration would result in a greater exposure to the bladder epithelial cells to the carcinogen by way of the urine than would be

possible by diffusion from the serum, through the subepithelial capillaries, across the basement membrane into the epithelium. The pharmacodynamics of urinary bladder carcinogenesis must be borne in mind when attempting to affect the process, as described elsewhere in this symposium (*39*).

However, beginning with experiments reported by Chapman et al. (*40*), evidence has accumulated suggesting that urine or substances in urine play a more direct role in bladder carcinogenesis than merely transporting carcinogens. In the experiments by Chapman et al. (*40*), the urinary bladder was divided in half and a pellet was inserted into each half. The dome half of the bladder did not have exposure to urine flow, whereas the lower half—the trigone portion—of the urinary bladder was exposed to urine by the usual flow from the ureters. The portion of the bladder exposed to urine and containing a pellet developed tumors, but the portion containing the pellet without exposure to urine did not develop tumors. As mentioned above, the role of the pellet in these experiments complicates the interpretation.

More direct evidence for a role of urine in bladder carcinogenesis was provided by a series of experiments by Oyasu and Rowland and their colleagues (*41-44*). If FANFT was administered to male Fischer rats for 14 weeks followed by control diet, the rats developed bladder tumors by 30 weeks of the experiment, as had been shown in several previous experiments by others (*8*). However, if the rats underwent bilateral ureterosigmoidostomies at the time FANFT was discontinued at the end of 14 weeks of administration, effectively diverting the flow of urine away from the bladder to the colon, a markedly decreased incidence of bladder tumors was induced (*41*). Thus, even though a supposedly carcinogenic dose of FANFT had been administered, it was necessary for urine to be present for expression of these tumors. Similarly, by using the heterotopic bladder model, a direct action for urine was again demonstrated (*42*). The bladder was exposed to 4 weeks of BBN administration and then transplanted to the back of another rat and attached to a reservoir through which various substances could be injected. These bladders developed tumors if urine was injected into them. However, if saline was injected instead of urine, a markedly decreased incidence of tumors resulted. The saline and urine were adjusted to be of equal pH and osmolality. Using an *in vitro* assay for ornithine decarboxylase activity and cell proliferation, Oyasu and his colleagues were able to demonstrate that most of the activity in urine was present in the molecular weight fraction greater than 10,000 daltons (*43*) and confirmed this finding in the heterotopic bladder model (*44*).

These experiments suggest that the early events in carcinogenesis of the urinary bladder can be induced by a variety of substances such as FANFT and that if they progress far enough, the urine will act as an enhancing substance, possibly by simply maintaining an increased proliferative rate of the bladder epithelium.

A Probabilistic Model of Urinary Bladder Carcinogenesis

The above experiments and others provide support for the hypothesis that carcinogenesis occurs in two steps. Because of the complexity, the interrelationships of the biological events are difficult to evaluate by use of *in vivo* models. Another approach is to examine specific questions in tissue culture systems. However, there are certain limitations within these systems also, which have been well documented in the literature. Another approach is to utilize a computer-based model which can analyze the interrelationships in mathematical terms and thus provide an aid in identifying critical experiments necessary for furthering our understanding of carcinogenesis.

We have devised a computer-based, probabilistic model of two-stage carcinogenesis (*45*) using data from the urinary bladder carcinogenesis experiments described above. However, the concepts and the basic model are applicable to other tumor systems as well. For this model, several assumptions had to be made concerning the carcinogenic process. The first is that the process requires at least two specific events which are irreversible. Secondly, these critical events can occur only during cell replication; they cannot occur when the cell is at rest (*25, 46*). Thirdly, the critical events occur only in stem cells in the urinary bladder. This could be a stem cell equivalent in other tissues. Our model is probabilistic. In the normal situation, each event is obviously rare.

The various possible cellular transitions are depicted in Figure 1. The critical transitions for carcinogenesis are from normal stem cell to initiated stem cell to transformed stem cell. The probability of a single normal cell becoming initiated during cell replication is given as p_{12}, and the probability of an individual initiated stem cell becoming a transformed stem cell is given as p_{23}. In the urinary bladder of the rat there are approximately 10^6 cells, and we estimate that approximately 10^5 are stem cells. The likelihood of developing a tumor within a specific period of time is thus a function of: the number of cells in each of the stem cell populations, the number of mitotic events in each stem cell popula-

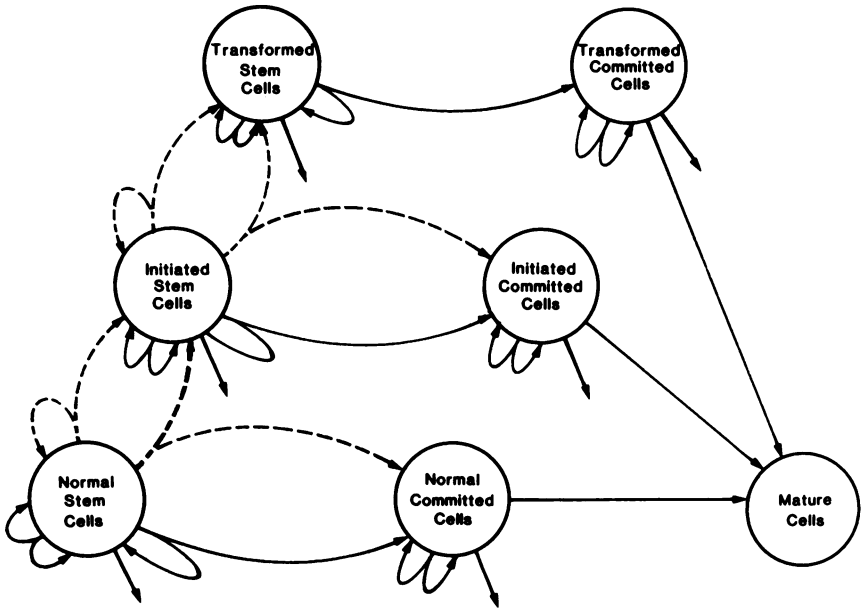

FIGURE 1. Possible cellular transitions.

tion during this time period and the probabilities p_{12} and p_{23}. The number of cells can be estimated by the histopathologic examination of the bladder at different time periods following various stimuli, such as FANFT or sodium saccharin, and the mitotic rate can be determined experimentally by autoradiography. Obviously, estimates need to be made of the transition probabilities, p_{12} and p_{23}. It should be recognized that the stimulus can cause any of the above parameters to change in value as a function of time.

In the FANFT Fischer rat model, it has been shown that hyperplasia (an increase in the number of cells) appears rapidly and gradually increases with time of administration of the chemical (*7, 8*). In addition, the mitotic rate increases. In the reversibility-irreversibility study of the various early lesions described above (*8-10*), it was also shown that the cell population sizes decrease if FANFT is withdrawn from the diet. The computer-based model reflects these changes over time for 1-day increments. By using the data from the above experiments as model input, predictions can be made of the rate of tumor formation and growth. Based on this model, we are able to show that p_{12} must increase dramatically above background levels during FANFT administration, but the transition probability p_{23} does not change from background. In fact, p_{23} must remain at background levels for the model to replicate empirically observed tumor incidences. Sodium saccharin was shown by the computer model not to alter either p_{12} or p_{23}. The entire effect of sodium saccharin can be explained on the basis of increased cell numbers (hyperplasia) and increased mitotic rate, which have been demonstrated experimentally (*29*).

Although this model involves two events in carcinogenesis, it is a more general formulation than the classical initiation and promotion concept described originally in mouse skin (*24, 25*). Thus, an increase in tumor incidence will occur by application of any agent which increases any of the critical variables: The number of normal stem cells, the mitotic rate of normal stem cells, the probability of the transition from normal to initiated stem cell (p_{12}), the number of initiated stem cells, the mitotic rate of initiated stem cells and the probability of the transition from initiated stem cell to transformed stem cell (p_{23}). Also, using such a model, one can demonstrate that the critical factors in most promotion experiments, including those in mouse skin with phorbol esters, involve an increase in the stem cell population and an increased mitotic rate rather than an actual change in p_{23}. The various properties of the classical initiation and promotion experiments, such as irreversibility of initiation, importance of sequence of administration and even the subdivision of promotion into various stages, can be readily explained with such a model. The importance of changes in stem cell population size and mitotic rates appears to have been underestimated.

We believe that synthesis of data from various

animal studies through computer-based modeling is a valuable, relatively low cost and rapid approach toward furthering the understanding of the complex interrelationship between multiple exposures to exogenous or endogenous agents and the development of tumors. Also, the availability of a model applicable to animals and man will assist in the extrapolation of animal data to human problems.

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REFERENCES

1. Price, J. M. Etiology of bladder cancer. In: Benign and Malignant Tumors of the Urinary Bladder (E. Maltry, Ed.), Medical Examination Publishing Co., Flushing, NY, 1971, pp. 189-251.
2. Clayson, D. B., and Cooper, E. H. Cancer of the urinary tract. Adv. Cancer Res. 13: 271-381 (1970).
3. Druckrey, H., Preussmann, R., Schmahl, D., and Svankovic, S. Organotrope carcinogene wirkungen bei 65 verschiedenen *N*-nitrosoverbindungen an BD-Ratten. Z. Krebsforsch 69: 103-201 (1967).
4. Erturk, E., Price, J. N., Morris, J. E., Cohen, S., Leith, R. S., VonEsch, A. M., and Crovetti, A. J. The production of carcinoma of the urinary bladder in rats by feeding *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res. 27: 1998-2002 (1967).
5. Hicks, R. M., and Wakefield, J. St. J. Rapid induction of bladder cancer in rats with *N*-methyl-*N*-nitrosourea. I. Histology. Chem. Biol. Interact. 5: 139-152 (1972).
6. Erturk, E., Cohen, S. M., Price, J. M., and Bryan, G. T. Pathogenesis, histogenesis, histology and transplantability of urinary bladder carcinomas induce in albino rats by oral administration of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide. Cancer Res. 29: 2219-2228 (1969).
7. Tiltman, A. J., and Friedell, G. H. The histogenesis of experimental bladder cancer. Invest. Urol. 9: 218-226 (1971).
8. Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H. Early lesions in experimental bladder cancer: scanning electron microscopy of cell surface markers. Cancer Res. 36: 2512-2517 (1976).
9. Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H. Light and scanning electron microscopy of exfoliated bladder epithelial cells in rats fed *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. J. Natl. Cancer Inst. 57: 63-66 (1976).
10. Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H. A long-term study of reversible and progressive urinary bladder cancer lesions in rats fed *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res. 37: 2817-2821 (1977).
11. Arai, M., Cohen, S. M., Jacobs, J. B., and Friedell, G. H. Effect of dose on urinary bladder carcinogenesis induced by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. J. Natl. Cancer Inst. 63: 1013-1016 (1979).
12. Cohen, S. M., Arai, M., Jacobs, J. B., and Friedell, G. H. Promoting effect of saccharin and DL-tryptophan in urinary bladder carcinogenesis. Cancer Res. 39: 1207-1217 (1979).
13. Fukushima, S., Friedell, G. H., Jacobs, J. B., and Cohen, S. M. Effect of L-tryptophan and sodium saccharin on urinary bladder carcinogenesis initiated by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide. Cancer Res. 41: 3100-3103 (1981).
14. Cohen, S. M., Murasaki, G., Fukushima, S., and Greenfield, R. E. Effect of regenerative hyperplasia in the urinary bladder carcinogenicity of sodium saccharin and *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res. 42: 65-71 (1982).
15. Tsuda, H., Miyata, Y., Murasaki, G., Kinoshita, H., Fukushima, S., and Ito, N. Synergistic effect of urinary bladder carcinogenesis in rats treated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide, *N*-2-fluorenylacetamide, and 3,3′-dichlorobenzidine. Gann 68: 183-192 (1977).
16. Tatematsu, M., Miyata, Y., Mizutani, M., Hananouchi, M., Hirose, M., and Ito, N. Summation effect of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, *N*-2-fluorenylacetamide, and 3,3′-dichlorobenzidine on urinary bladder carcinogenesis in rats. Gann 68: 193-202 (1977).
17. Bryan, G. T., and Springberg, P. D. Role of the vehicle in the genesis of bladder carcinomas in mice by the pellet implantation technic. Cancer Res. 26: 105-109 (1966).
18. Clayson, D. B., Lawson, T. A., and Pringle, J. A. S. The carcinogenic action of 2-aminodiphenylene oxide and 4-aminodiphenyl on the bladder and liver of the C57 X IF mouse. Brit. J. Cancer 21: 755-762 (1967).
19. Jull, J. W. Carcinogenesis by chemicals implanted into the bladder: an evaluation. In: Recent Topics in Chemical Carcinogenesis (S. Odashima, S. Takayama, and H. Sato, Eds.), University of Tokyo Press, Tokyo, 1975, pp. 383-392.
20. Hicks, R. M., and Chowaniec, J. The importance of synergy between weak carcinogens in the induction of bladder cancer in experimental animals and humans. Cancer Res. 37: 2943-2949 (1977).
21. Nakanishi, K., Hagiwara, A., Shibata, M., Imaida, K., Tatematsu, M., and Ito, N. Dose response of saccharin in induction of urinary bladder hyperplasias in Fischer 344 rats pretreated with *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. J. Natl. Cancer Inst. 65: 1005-1010 (1980).
22. Nakanishi, K., Hirose, M., Ogiso, T., Hasegawa, R., Arai, M., and Ito, N. Effects of sodium saccharin and caffeine on the urinary bladder of rats treated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Gann 71: 490-500 (1980).
23. Swaminathan, S., and Lower, G. M., Jr. Biotransformations and excretions of nitrofurans. Carcinog. Compr. Surv. 4: 59-98 (1978).
24. Boutwell, R. K. Some biological aspects of skin carcinogenesis. Progr. Exptl. Tumor Res. 4: 207-250 (1964).
25. Scribner, J. D. and Susa, R. Tumor initiation and promotion. Int. Rev. Exptl. Pathol. 18: 137-198 (1978).
26. Cancer Testing Technology and Saccharin. Office of Technology Assessment, United States Printing Office, Washington, DC, 1977.
27. Moore, C. W. and Schmick, A. Genetic effects of impure and pure saccharin in yeast. Science 205: 1007-1010 (1979).
28. Kinsella, A. R., and Radman, M. Tumor promoter induces sister chromatid exchanges: relevance to mechanisms of carcinogenesis. Proc. Natl. Acad. Sci. (U.S.) 75: 6149-6153 (1978).
29. Fukushima, S., and Cohen, S. M. Saccharin-induced hyperplasia of the rat urinary bladder. Cancer Res. 40: 734-736 (1980).
30. Radomski, J. L., Glass, E. M., and Deichman, W. B. Transitional cell hyperplasia in the bladders of dogs fed D,L-tryptophan. Cancer Res. 31: 1690-1694 (1971).
31. Tiltman, A. J., and Friedell, G. H. Effect of feeding *N*-[4-

(5-nitro-2-furyl)-2-thiazolyl]formamide on mitotic activity of rat urinary-bladder epithelium. J. Natl. Cancer Inst. 48: 125-129 (1972).
32. Shirai, T., Cohen, S. M., Fukushima, S., Hananouchi, M., and Ito, N. Reversible papillary hyperplasia of the rat urinary bladder. Am. J. Pathol. 81: 33-48 (1978).
33. Fukushima, S., Arai, M., Cohen, S. M., Jacobs, J. B., and Friedell, G. H. Scanning electron microscopy of cyclophosphamide-induced hyperplasia of the rat urinary bladder. Lab. Invest. 44: 89-96 (1981).
34. Fukushima, S., Cohen, S. M., Arai, M., Jacobs, J. B., and Friedell, G. H. Scanning electron microscopy of reversible hyperplasia of the rat urinary bladder. Am. J. Pathol. 102: 373-380 (1981).
35. Arnold, D. L., Moodie, C. A., Grice, H. C., Charbonneau, S. M., Stavric, B., Collins, B. T., McGuire, P. F., Zawidska, Z. Z., and Munro, I. C. Long-term toxicity of *ortho*-toluenesulfonamide and sodium saccharin in the rat. Toxicol. Appl. Pharmacol. 52: 113-152 (1980).
36. Bryan, G. T., Erturk, E., and Yoshida, O. Production of urinary bladder carcinomas in mice by sodium saccharin. Science 168: 1238-1240 (1970).
37. Klemencic, J. M., and Wang, C. Y. Mutagenicity of nitrofurans. Carcinog. Compr. Surv. 4: 99-130 (1978).
38. Mohn, G. R., and Ellenberger, J. Genetic effects of cyclophosphamide, ifosfamide and trafosfamide. Mutat. Res. 32: 331-360 (1976).
39. Zenser, T. V., Cohen, S. M., Mattamal, M. B., Wise, R., and Davis, B. B. Prostaglandin hydroperoxidase-catalyzed activation of certain *N*-substituted aryl renal and bladder carcinogens. In press.
40. Chapman, W. H., Kirchkin, d., and McRoberts, J. W. Effect of the urine and calculus formation in the incidence of bladder tumors in rats implanted with paraffin wax pellets. Cancer Res. 33: 1225-1229 (1973).
41. Rowland, R. G., Henneberry, M. O., Oyasu, R., and Grayhack, J. T. Effects of urine and continued exposure to carcinogen on progression of early neoplastic urinary bladder lesions. Cancer Res. 40: 4524-4527 (1980).
42. Oyasu, R., Hirao, Y., and Izumi, K. Enhancement by urine of urinary bladder carcinogenesis. Cancer Res. 41: 478-481 (1981).
43. Izumi, K., Hirao, Y., Happ, G., and Oyasu, R. *In vitro* induction of ornithine decarboxylase in urinary bladder carcinoma cells. Cancer Res. 41: 405-409 (1981).
44. Babaya, K., Miyata, Y., Chmiel, J. S., and Oyasu, R. Effects of rat urine fractionated by molecular weight on urinary bladder carcinogenesis. Cancer Res. 42: 15-18 (1982).
45. Greenfield, R. E., Ellwein, L. B., and Cohen, S. M. A computer-based probabilistic model of carcinogenesis and analysis of experimental urinary bladder carcinogenesis induced by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide and sodium saccharin. Carcinogenesis, submitted for publication.
46. Warwick, G. P. Effect of the cell cycle on carcinogenesis. Fed. Proc. 30: 1760-1765 (1971)